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GRANT NO: N00014-89-J-3070
Title: Characterization of Ground Squirrel Retinal Ganglion Cells

PROGRESS REPORT (Covers period 7-1-90 to 6-30-90)

PERSONNEL:

Name	Title	From	To	% Effort
N. Lugo-García, Ph.D.	P.I.	7-1-89	6-30-91	25
R. E. Blanco, Ph.D.	Co-I.	7-1-89	6-30-91	20
Ivonne Santiago	Technician	5-1-90	6-30-91	100

Our work during this period mainly involved the use of: 1) immunohistochemical techniques to identify neuroactive substances in the ground squirrel retina (with emphasis on the substances present in ganglion cells), 2) intracellular injections of Lucifer Yellow and retrograde transport of fluorescent carbocyanine dyes to examine the dendritic morphology of ganglion cells projecting to the superior colliculus and dorsal lateral geniculate nucleus, and 3) EM studies of retinas from the immunohistochemical experiments to determine the synaptic organization in the inner plexiform layer of neurons expressing a particular neuroactive substance.

1) Our studies of GAD-like and GABA-like immunoreactivity revealed labeling in an heterogeneous population of neurons in the ganglion cell layer. To determine if some of the immunoreactive cells in this layer were ganglion cells, double-labeling experiments were performed using rhodamine latex microspheres ("beads") as a retrograde neuronal tracer. These experiments revealed a small population of GAD-positive ganglion cells which project to the superior colliculus[1].

Retinas incubated with an antibody against TH had labeled amacrine and displaced amacrine cells. We studied the morphology, location, soma size distribution and distance to the nearest neighbor of these TH-amacrines. These results were presented at the annual meeting of the American Association of Anatomists on April, 1991[2].

Idamaris Santiago, a medical student, and Marizabel La Puerta, a graduate student working in our laboratory during the summer months, are doing some additional screening of neuroactive substances in the ground squirrel retina. They are conducting experiments with antibodies against substance P, glycine, somatostatin, serotonin and CRF.

2) Walter Nieves, another medical student presently working in our laboratory, is determining the dendritic morphology of ganglion cells projecting to the superior colliculus and the dorsal

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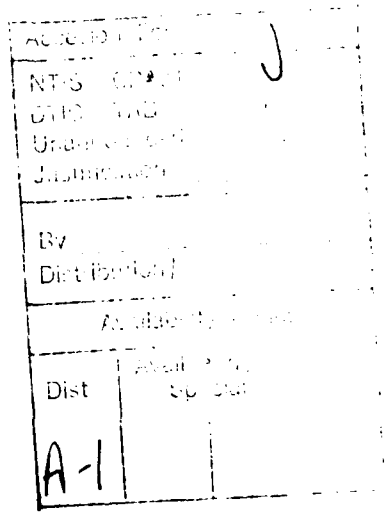
lateral geniculate nucleus. We have encountered some technical problems when performing the intracellular injections of Lucifer Yellow and have ordered a "Micro-g vibration isolation system" to deal with the problems. In the meantime, Mr. Nieves has been labeling ganglion cells with diI. The dye has been deposited into the retinal target nuclei both in fixed and fresh tissue and we are in the process of determining optimal transport time of the dye.

3) We have been successful in devising a fixation protocol which is compatible with our immunohistochemistry and EM techniques. We are presently working on the synaptic organization in the inner nuclear layer of GAD-positive ganglion cells and TH-positive amacrines.



[1] Lugo-García, N. and Blanco, R.E., (1991) Localization of GAD- and GABA-like immunoreactivity in ground squirrel retina: Retrograde labeling demonstrates GAD-positive ganglion cells. Brain Res. (in press).

[2] Lugo-García, N. and Blanco, R.E., (1991) Amacrine cells immunoreactive to tyrosine hydroxylase in the ground squirrel retina. Anat. Rec. 229:56A.